SYBR NGS Library Quantification Kit for Illumina

Project number: S665948

Storage condition: -20℃, 12 months, if need to be used frequently, can be stored in 2-8℃, try to avoid repeated freezing and thawing.

Product content

Component	S665948-1ml	S665948-5ml
2×SYBR qPCR Master Mix	1ml	5 x 1ml
qPCR Primer Mix	100μl	5 x 100μl
DNA Standard 1	100μl	5 x 100μl
DNA Standard 2	100μl	5 x 100μl
DNA Standard 3	100μl	5 x 100μl
DNA Standard 4	100μl	5 x 100μl
DNA Standard 5	100μl	5 x 100μl
50 x High ROX	40μl	200μl

Product Introduction

This product is used for real-time fluorescence quantitative PCR (qPCR) using the product after NGS library construction by dye method (SYBR Green I). The kit provides the reaction mixture, DNA primer mixture, and standards required for the qPCR process, and the reagent system is complete, easy and convenient to operate. The kit uses a new chemically modified high-efficiency hot-start polymerase, the activation of the enzyme needs to be incubated at 95 ℃ for 10 min. the product is highly specific, high amplification efficiency, and able to quickly and accurately quantify the concentration of the constructed library. It is suitable for fluorescent quantitative PCR instruments that do not require ROX as a calibration dye, such as Roche LightCycler 480, Roche LightCyler 96, Bio-radiCyleriQ, iQ5, CFX96.

ROX dye is used to correct the fluorescence signal error generated between wells of a quantitative PCR instrument, and is generally used in Real Time PCR amplifiers from ABI, Stratagene, and other companies. The excitation optics vary from instrument to instrument, so the concentration of ROX dye must be matched to the corresponding fluorescence quantitative PCR instrument.

Instruments that do not require ROX calibration: Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, etc.

Instruments requiring Low ROX calibration: ABI Prism7500/7500 Fast, QuantStudio®3 System, QuantStudio®5 System, QuantStudio®6 Flex System, QuantStudio®7 Flex System, ViiA 7 System, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, and others.

Instruments requiring High ROX calibration: ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Note: High Rox and Low Rox are formulated as described in Use 2.

Scope of application

This product is designed for absolute quantification of the concentration of Illumina platform second-generation sequencing libraries. The end of the library contains Illumin P5 and P7 chip binding sequences, the length of which does not exceed 1kb, and the concentration of which is not less than 0.002pM can be used to perform quantitative experiments with this product. The qPCR Primer Mix provided in the kit contains the following two primer sequences:

Primer 1:5'-AAT GAT ACG GCG ACC ACC GA-3' Primer 2: 5'-CAA GCA GAA GAC GGC ATA CGA-3'
The primer sequence can be used in advance to confirm whether the library can be amplified by that primer pair.

Usage

Amplification template preparation
The library samples to be detected were diluted with TE (10 mM Tris-Cl, pH 8.0, 1 mM EDTA), and the concentration after dilution was as close as possible to the range of 0.01-20 pM. 4° C on ice was set aside.
qPCR reaction system preparation
The desired cryopreservation reagent is pre-melted completely and mixed by inverting several times before preparation, then centrifuged briefly and set aside.
The base reaction system for 20 μl was as follows:

reagents	20μl reaction system
2×SYBR qPCR Master Mix	10μl
qPCR Primer Mix 1	0.8μl
Template	4 μl
ddH_2O	5.2μl

Description: High Rox model: add 1 μl High Rox per 50 μl of reaction system; Low Rox model: 1 μl High Rox per 500 μl of reaction system.
Prepare a sufficient amount of reaction system mixture according to the need, mix well and add to the reaction wells in a volume of 16 μl per well, add the same volume of TE to the blank control, and then add the prepared standards and diluted samples to the corresponding reaction wells in a volume of 4 μl/well. It is recommended to use 20 μl reaction system, if you need to carry out a smaller system reaction, the system components can be reduced in equal proportion.
qPCR reaction program

steps	temperature	time	cycles
Pre denaturation	95° C	10min	1
denaturation	95° C	10sec	40
Annealing/Extension	62° C	30sec	
Dissolution curve analysis		65-95° C	

The annealing temperature should be 60-64° C as a reference for the setting range, and the annealing temperature can be increased when a non-specific reaction occurs.
If the average length of the library is greater than 700bp, the annealing/extension time should be increased appropriately.

data analysis

Standard curve production
The standard curve was plotted using Ct values in the valid range. The standard curve correlation coefficient R2 should not be less than 0.99 and the slope should lie between -3.1 and -3.6. If the standard curve parameters are not reasonable, it is recommended to repeat the experiment.

DNA Standard name	DNA Standard concentration
DNA Standard 1	20pM
DNA Standard 2	2pM
DNA Standard 3	0.2pM
DNA Standard 4	0.02pM
DNA Standard 5	0.002pM

Library Concentration Calculations

The difference in Ct between the three replicate wells of the experiment should be no more than 0.2, otherwise the invalid data should be deleted or the experiment should be repeated. Do not use the Ct outside the valid Ct range of the standard curve to calculate the concentration of the diluted libraries. Please refer to the data processing Excel of this product for the specific library concentration calculation method.

matters needing attention

These instructions should be read in detail before testing. It should be carried out by personnel with specialized experience or qualified by training.

Mix gently by turning up and down, avoid foaming as much as possible, and centrifuge for a short time before use.

Avoid repeated freezing and thawing of this product; repeated freezing and thawing may degrade product performance.

When preparing reaction solutions, use new or non-contaminated tips and centrifuge tubes to prevent contamination as much as possible.